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(71) Applicant: SHANBROM TECHNOLOGIES LLC [US/US]; Suite B, 603 West Ojai Avenue, Ojai, CA 93023-3732 (US).

(72) Inventor: SHANBROM, Edward; 2252 Liane Lane, Santa Ana, CA 92705 (US).

(74) Agents: KIRCHANSKI, Stefan, J. et al.; Hogan & Hartson L.L.P., Biltmore Tower, 500 South Grand Avenue, Suite 1900, Los Angeles, CA 90071 (US).

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(54) Title: ANTIMICROBIAL LEES

(57) Abstract: The lees or "dregs" produced during wine making are rich sources of antioxidants. Unexpectedly, these materials show significant antibacterial properties as well as antioxidant properties. The lees of red wine which consist of tannins and plant pigments precipitated around crystals of potassium tartarate can advantageously be used directly as a tonic or demulcent. The material can also be used topically for disinfecting the skin, etc. In addition, it is possible to use organic polymers to bind the pigments and/or solubilize them from the tartaric salt to facilitate their use or to make a relatively pure pigment/tannin component.

ANTIMICROBIAL LEES

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present application concerns natural products and more especially valuable materials that can be derived from the byproducts of vinification

2. Description of Related Art

Currently there is a growing concern on the part of the public that our modern diet of highly processed and refined foods is "missing some essential components" necessary for health and well-being. This "natural food" movement probably derives from at least two sources. First is the discovery of vitamins over the last three quarter's of a century, and the public realization that consumption of apparently adequate food can actually result in a serious deficiency syndrome. It is not hard to imagine that the already discovered vitamins, which are now added back to our refined foods, are but the tip of the iceberg. That is, many other vitamin-like substances may remain to be discovered meaning that our food is presently dangerously deficient in essential nutrients.

Second is the realization that consumption of certain foods—in particular animal fats—seems to result in significant heart and vascular disease. Not only has the public come to learn that apparently complete foods are lacking a key ingredient, but the public has also learned that apparently innocuous and much favored foods are actually silent killers. The question in the public mind is "why did fatty foods suddenly become so deadly?" One answer is that fatty foods have always been harmful but that people didn't used to consume so much of them. Another answer is that lack of physical activity exacerbates the damage caused by fatty food—the American public certainly appears to have grown more sedentary as compared to Americans a century ago. However, the picture is convoluted by certain groups of people that appear to be immune to the dangers of fatty diets.

For example, some Europeans, particularly of Mediterranean origin, appear to consume diets high in fatty foods with little or no medical consequences. Some experts believe this appearance is actually an artifact of relative recent dietary changes that have not yet "caught up" with Europeans. Under this scenario incidence of heart disease should soon increase sharply in those European areas. Other experts believe that "good fat" (e.g., monounsaturated olive oil) neutralizes the other fat in the European diet. This has sparked an olive oil fad in the United States. While the result is undoubtedly widespread culinary improvement, there is as yet no evidence of positive medical consequences. Finally, there is the "red wine connection": many Europeans consume a considerable quantity of red wine, and some experts have opined that a constituent in red wine acts to neutralize the deleterious effects of a fatty diet.

Although some believe that the alcohol in wine is the source of its apparently beneficial properties, it does appear that red wine is more beneficial than white wine. Since both drinks have about the same level of alcohol, one naturally comes to suspect that the coloring component of the wine is the source of the beneficial properties. It is known that the polyphenolic pigments and tannins present in red wine (but largely missing from white wine) are powerful antioxidants. There is already something of an "antioxidant fad" going on in the field of dietary supplements where many people are consuming vitamin A, vitamin C, vitamin E, and various plant polyphenols in hopes of reducing oxidative damage and the presumed aging effects thereof. Thus, the antioxidant properties of red wine falls right into place with this trend although it has not been proven that antioxidant *per se* are the source of the beneficial properties of red wine.

Therefore, there is a considerable need for providing the apparent benefits of red wine without increasing the public's consumption of alcohol and at a fairly modest expense.

SUMMARY OF THE INVENTION

The present invention involves the discovery that the lees or "dregs" produced during wine making are rich sources of antioxidants. Unexpectedly these materials also show significant antibacterial properties as well as antioxidant properties. The lees of red

wine which consist of tannins and plant pigments precipitated around crystals of potassium tartarate can advantageously be used directly as a tonic or demulcent. The material can also be used topically. In addition, it is possible to use organic polymers to bind the pigments and/or solubilize them from the tartaric salt to facilitate their use or to make a relatively pure pigment/tannin component.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a flow diagram of lees separation.

DETAILED DESCRIPTION

OF THE PREFERRED EMBODIMENTS

The following description is provided to enable any person skilled in the art to make and use the invention and sets forth the best modes contemplated by the inventor of carrying out his invention. Various modifications, however, will remain readily apparent to those skilled in the art, since the general principles of the present invention have been defined herein specifically to provide useful compositions from wine lees.

It is not known how the beverage known as wine was first discovered. Suffice it to say that wine goes far back in human history being known from all the classical civilizations that surround the Mediterranean. However, it is clear that wine and wine making go back far beyond those classical civilizations. It is likely that wine making and viticulture (growing of grapes) reaches back well into the New Stone Age and possibly into the Old Stone Age. Many people think that wine is merely fermented grape juice. This description might fit white wine but is certainly incorrect for red wine, the primary beverage of antiquity. This is so because the pigments and tannins that give red wine its color and characteristic flavor are not present in grape juice. The grape skins must be fermented with the juice until the mixture contains sufficient alcohol to liberate the color from the skins. Only then can the skins be pressed to wring out the nascent vintage after which the skins and seeds can be discarded as pommace.

It is not recorded what fortunate person first discovered the transfer of color from skins to wine, but this discovery was essential for the production of wine as a safe and stable beverage. Not only do the pigments and tannins give flavor and color to the wine, they protect the beverage from oxidation and spoilage. It is not likely that wines in the ancient world were normally stored for more than one year. As soon as the fermentation was complete, the wines were sealed in jars and shipped for immediate consumption. Little wonder that wine was generally cut with water or sweetened with sugar of lead as those wines were undoubtedly harsh and tannic.

Later in history, probably in the Middle Ages, it was discovered that under proper conditions wines could be stored for many years and actually improve in sensory quality and drinkability. It was then, when wines were stored, that lees, dregs or sediments first came to be noticed. As wine ages, a slow chemical change occurs. Part of this is due to controlled oxidation as tiny amounts of air seep into the wine. In any case, the pigments and tannins polymerize and tend to fall out of solution. Organic fruit acids such as tartaric acid also precipitate from solution particularly at lower temperatures. This accounts for the dark sediment or lees found in wine barrels and to a lesser extent in wine bottles. This material is removed by filtration or decanting since it renders the wine cloudy and is unpleasant to swallow if present in large quantities.

From time to time some practical use has been made of the lees. They are the primary source of potassium tartarate (cream of tartar), and at one time the lees were extracted to yield a purple pigment used to mark meats and other foods. Nevertheless, the vast majority of lees are simply discarded or returned to the vineyard as a sort of soil amendment. Now I have discovered a number of uses for these materials based on their antioxidant and hitherto unrecognized antimicrobial properties. I have also devised a method for handling the lees to simplify their use.

Lees are obtained from the winery as a suspension of solid material in a quantity of wine. As explained above, the solid material is largely potassium tartarate coated with polymerized tannins and pigments. The first step of my process is to separate the liquid wine from the solid lees. Initial stages of this separation are achieved by gravity decanting and/or by centrifugation. Simple filtration is generally not effective because

the almost colloidal lees tend to plug the filter. Once the sediment is largely concentrated, crosslinked polyvinylpyrrolidone (xPVP) is added to the supernatant. This material captures the colors and tannins so that the pigment concentrate can be used as explained later. After the xPVP is removed by filtration or centrifugation, the essentially colorless supernatant can then be flash distilled to recover the ethanol as a byproduct. An outline of this process is shown in Fig. 1. A suspension of lees in wine 10 is obtained from the winery. In a separation step 20 a sediment component 70 is separated from a supernatant component 30 preferably by gravitational means such as decanting or centrifugation. Crosslinked polyvinylpyrrolidone 40 (xPVP) is mixed into the supernatant to capture essentially all of the pigment/tannins 50 which are separated gravitationally. A colorless supernatant 60 that results consists largely of water, alcohol and carbohydrates. The supernatant 60 can advantageously be distilled or otherwise treated to recover the ethyl alcohol. The sediment 70 can be used directly or, as detailed below, can be further fractionated, as with soluble PVP, to liberate the pigments/tannins which can be combined with the pigment/tannins 50 purified from the supernatant 30. Other solid materials can be used to bind the pigment/tannins and can thus be substituted for xPVP. One such material that has worked well in the processes of the present invention is cholestyramine.

Additional Purification

Most attempts to dissolve the purified lees were unsuccessful. Solvents can be used to extract some of the bound pigment but were generally not very effective. For example, sedimented lees 70 were suspended in 5% dextrose, 20% ethanol (ETOH), distilled water, 1.0M sodium chloride or 0.9% sodium chloride (physiological saline). Some color was dissolved by the distilled water, but the salt solutions were essentially ineffective. Dextrose and ethanol were moderately effective at removing color from the lees.

There are at least two simple effective ways to extract the pigments/tannins from the lees (mostly tartarate crystals). If a suspension of lees are carefully brought to an alkaline pH by drop wise addition of sodium hydroxide, the solid material (tartarate)

goes into solution. After this xPVP can be added to precipitate the pigments. The other approach is to add soluble PVP to a suspension of the lees in water. With continued mixing the PVP will bring the pigments/tannins into solution after which the largely colorless tartarate can be removed by centrifugation. The pigments/tannins can be extracted from the soluble PVP if a PVP-free product is desired (e.g., with butanol) or xPVP can be used to render the pigmented components insoluble.

Antioxidant Measurements

Earlier I developed an iodine-based method for measuring antioxidant levels. This method forms the subject of copending application Serial No. 09/315,688, filed May 29, 1999 and entitled "Method for Quantifying Antioxidant Levels In Foods and Medical Specimens" which is incorporated herein by reference. Briefly, an aliquot of PVP-iodine is added to each sample and reduction of the iodine to iodide was followed with an iodide electrode. Antioxidant units represent the normalized quantity of reduced iodine and are called Iodine Reducing Units (IRU). The measurements were made by taking 4 g aliquots of lees 70 and suspending the aliquots in 25 ml of water to which the iodine reagent is added. The "straight" lees were modified with additives to determine additive effect on the antioxidant determination. The treatments were as follows: a) "straight" lees; b) addition of an equivalent weight of xPVP prior to suspension and addition of iodine; c) addition of 25% soluble PVP (e.g. 1 g; and d) addition of 50% soluble PVP (e.g. 2 g). The results are shown in Table 1.

Table 1.

Treatment	IRU
a)	972
b)	725
c)	825
d)	1120

Apparently the added xPVP effectively captures some of the pigment/tannin material and prevents it from reacting with the iodine. On the other hand, the addition of soluble PVP, particularly at the higher concentration, appears to liberate the antioxidant material and facilitate its reaction with the iodine.

Additional antioxidant measurements were made of 1g aliquots of additional grape-derived materials as follows: a) xPVP extract from concord grape juice; b) 50% soluble PVP pigment solution extracted from the material of a); c) lees sediments from merlot wine; and d) lees sediments from concord grape wine. The results are show in Table 2.

Table 2.

Treatment	IRU
a)	384
b)	672
c)	977
d)	1590

From these results we can see that the wine lees have very high antioxidant levels as compared to materials extracted from grape juice. Again, higher readings are obtained in the presence of soluble PVP. It appears that concord grape wine lees have a somewhat higher level of antioxidants than do vinifera grape (European grape) lees.

Antibacterial Properties of Lees

I have previously discovered that pigment materials extracted from certain fruit juices such as cranberry juice have unexpected antibacterial properties. However, those studies also showed that many fruit juices, such as grape juice, were essentially devoid of antibacterial properties. Therefore, I was surprised to discover that wine lees have significant antibacterial properties. Apparently there is some transformation during the

vinification process that augments antibacterial properties. Alternatively, the yeasts contribute antibacterial substances or the entire process concentrates a grape antibacterial substance. Another consideration is that most fruit juices are heated (pasteurized) during processing. It is possible that the antibacterial substances are heat labile. It is my understanding that must for wine production is rarely pasteurized. The antibacterial tests were performed on suspensions ($1x10^3$ bacterial/ml) of *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus*, or *Bacillus subtilis*. One gram of each substance to be tested was suspended in an equal weight of water to produce a 1:2 dilution. Then successive two-fold dilutions were made (1:4, 1:8, 1:16, 1:32, 1:64, 1:128, etc.). One ml of each dilution was added to an equivalent volume of bacteria suspension and incubated at room temperature for 30 min. After this the solution was streaked on nutrient agar and incubated under conditions favorable for bacterial growth. The titer of a material represents the highest dilution that completely inhibited bacterial growth.

The following materials were tested: a) fresh (wet) merlot lees sediment 70; b) dried merlot lees sediment 70; c) lees sediment 70 mixed with 25% (by weight) xPVP; and d) lees sediment 70 mixed with 25% (by weight) soluble PVP. The results for each bacterial species are presented in Table 3.

Table 3.

E. coli		*P. aeruginosa*	
a)	1:128	a)	1:128
b)	1:32	b)	1:16
c)	1:32	c)	1:16
d)	1:64	d)	1:16

S. aureusi		*S. epidermitis*	
a)	1:128	a)	1:128
b)	1:32	b)	1:32
c)	1:32	c)	1:32
d)	132	d)	1:64

B. subtilis	
a)	1:64
b)	1:16
c)	1:16
d)	1:32

These results show that the lees sediment contains a powerful antimicrobial agent. It appears that solubility of this agent has a significant effect on the overall results. Once the lees have dried the agent is significant less soluble. It is possible that drying destroys the agent, but in most cases activity is partly restored by 25% soluble PVP. This is similar to the results with the antioxidant measurements where soluble PVP increased the availability of the antioxidant material. It appears that the PVP stabilizes and increases the solubility of the antibacterial property of the lees. It is believed that a higher concentration of soluble PVP would completely restore the antibacterial properties of the dried lees sediment.

Significantly, antibacterial measurements made on the concord grape juice "warm tank bottoms" showed very high antioxidant levels showed little antibacterial properties. The "tank bottoms" showed an antioxidant value of 2360 ITU when measured as explained above. In spite of this extremely high antioxidant value the antimicrobial titer against *E. coli* was only 1:4. On the other hand, lees sediment from concord grape wine showed considerable antibacterial properties. Again, some aspect of the vinification process appears to contribute to antimicrobial properties but not to antioxidant properties, or perhaps heating destroys the antibacterial properties..

Antioxidant Tonic/Demulcent

The sediment fraction (tartarate plus pigments) can be processed further to purify the pigments or may be used directly. I have found that this material can be ingested directly as a tonic or demulcent. There is a long history of using potassium tartarate in this fashion. The addition of the polymerized tannins and pigments enhance this effect. This is probably because of the antimicrobial properties of these substances (below).

Also, these antioxidant materials are readily absorbed after ingestion as can be monitored as an increase in the antioxidant properties of excreted urine. For example, I ingested approximately 10 g of lees sediment 70 taking samples of my urine both before and after ingestion. Ten ml. samples of urine were tested for antioxidant content using an iodine-based method explained above. By this method it was determined that urine prior to ingestion of the lees had a reading of 278 IRU. After two hours the urine showed 667 IRU; after three additional hours the value had decreased to 518 IRU. This demonstrates that an antioxidant component is readily absorbed from the lees and excreted in the urine. Therefore, there is a significant amount of this material circulating in the blood prior to excretion. With the recent discovery that atherosclerosis may be due to chronic circulating bacterial infections, it is tempting to speculate that the beneficial effects of red wine are at least partially due to the antibacterial substance whose discovery is described above.

Antibacterial Gloves

In checking these materials for antimicrobial properties, I came upon an unexpected use for them. I wished to determine whether these materials have any effect on the bacterial flora of the human hand. I "powdered" a latex glove with finely powdered lees prepared as described. Both hands were sampled for skin bacteria (swab plated on nutrient agar); then a normal powdered latex glove was placed on one hand while a lees powdered glove was placed on the other. After 60 min of wear, the gloves were removed and a second bacteriological sample was taken. As shown in Table 4, the lees completely prevented bacterial growth. The number of plus signs ("+") indicates the amount of bacterial growth.

Table 4

	Latex Control Glove	Lees Treated Glove
Start	+++	+++
After 60 minutes	+++	No Growth

In addition to the equivalents of the claimed elements, obvious substitutions now or later known to one with ordinary skill in the art are defined to be within the scope of the defined elements. The claims are thus to be understood to include what is specifically

illustrated and described above, what is conceptually equivalent, what can be obviously substituted and also what essentially incorporates the essential idea of the invention. Those skilled in the art will appreciate that various adaptations and modifications of the just-described preferred embodiment can be configured without departing from the scope the invention. It is to be understood that, within the scope of the appended claims, the invention may be practiced other than as specifically described herein.

CLAIMS

What Is Claimed Is:

1. A topical antimicrobial material comprising lees formed during the vinification of grape juice.

2. An antimicrobial glove comprising a glove powdered on an inner surface with the antimicrobial material of Claim 1.

3. A method of treating skin comprising application of an effective quantity of lees produced by vinification of grape juice, said quantity effective at killing skin bacteria.

4. A method for digestive system treatment comprising the ingestion of lees formed during the vinification of grape juice.

5. An antibacterial substance produced by separating pigmented materials from tartarate of lees formed during the vinification of grape juice wherein the antibacterial substance is present in the pigmented materials.

6. The antibacterial substance of Claim 5, wherein soluble polyvinylpyrrolidone is used to separate the pigmented materials from the tartarate.

7. The antibacterial substance of Claim 5, wherein crosslinked polyvinylpyrrolidone is used to separate the pigmented materials from the tartarate.

8. The antibacterial substance of Claim 5, wherein cholestyramine is used to separate the pigmented materials from the tartarate.

9. A method of producing an antibacterial substance from lees produced by vinification of grape juice comprising the steps of:

separating pigmented solid material from a supernatant fraction;

contacting the pigmented solid material with a binding material which binds pigments and tannins; and

separating the binding material from the solid material whereby the antibacterial substance is transferred to the binding material.

10. The method of Claim 9, wherein the binding material is selected from the group consisting of cholestyramine, crosslinked polyvinylpyrrolidone and soluble polyvinylpyrrolidone.

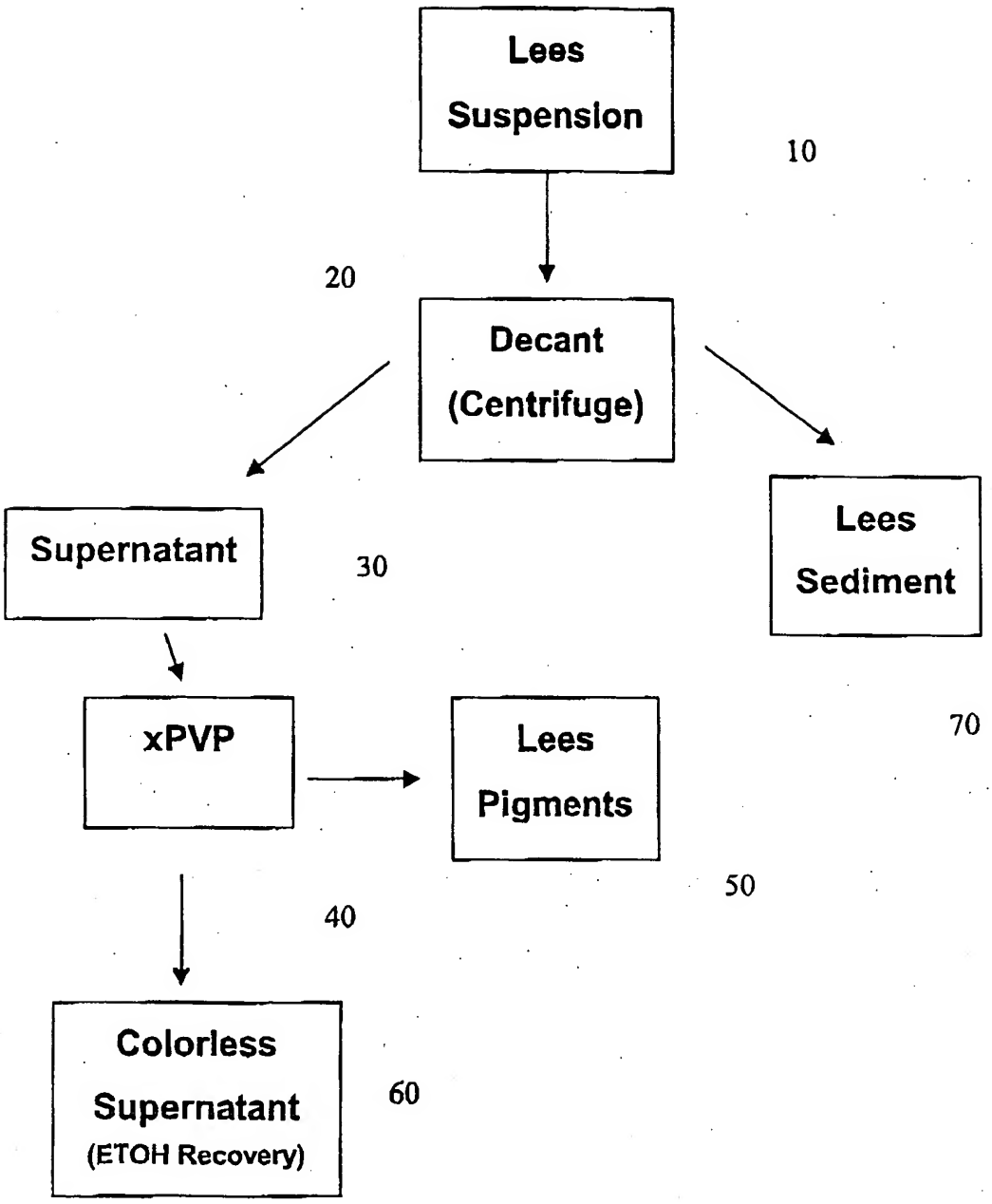
Lees Suspension
10
20
Decant (Centrifuge)
Supernatant
30
Lees Sediment
xPVP
Lees Pigments
70
50
40
Colorless Supernatant (ETOH Recovery)
60

Fig. 1

INTERNATIONAL SEARCH REPORT

International Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A01N65/00 A61K35/78 A61L31/16 A61B19/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A01N C12H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, BIOSIS, CHEM ABS Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	page 1 -page 2, line 14 page 2, line 58 -page 3, line 14; claims; examples 5,6	9,10

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[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance
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'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
'O' document referring to an oral disclosure, use, exhibition or other means
'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

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